

What is claimed is:

1. A method for predicting peak width of a solute peak in a gradient elution chromatography program, wherein the method comprises:

- 5 i) performing a time segmented numerical analysis,
wherein, within a given time segment, a strong component is presumed present in an amount that is constant;
- ii) calculating contribution to broadening of the solute peak in the given time segment;
- 10 iii) correcting accumulated peak width for peak compression occurring when the amount of strong component relative to weak component changes during the chromatography program;
- iv) incrementing the amount of the strong component to its next value in a successive time segment;
- 15 v) repeating steps i-iv until the solute peak elutes; and
- vi) optionally displaying the accumulated peak width of the solute peak.

2. The method of claim 1, further comprising:

- vii) repeating steps i-vi) for at least one successive solute peak.

3. The method of claim 1 or 2, wherein accumulated peak width at the given time segment is calculated according to an equation selected from the group consisting of:

$$\sigma_{current\ total} = \left(\left(\sigma_{previous\ total} * \left(1 - \frac{\frac{1}{1+k_{current\ segment}} - \frac{1}{1+k_{previous\ segment}}}{1 - \frac{1}{1+k_{previous\ segment}}} \right) \right)^2 + \sigma_{current\ segment}^2 \right)^{1/2}, \text{ wherein}$$

25 k represents retention factor, and σ represents peak standard deviation expressed as distance; algebraic equivalents thereof; an equation which can be transformed, using

known identities from chromatographic theory, into an algebraic equivalent thereof; and derivations thereof wherein peak standard deviation is expressed as time or as volume.

4. The method of claim 1, wherein the gradient is selected from the group
5 consisting of linear gradients, non-linear gradients of any shape, step-wise changes in mobile phase compositions, combinations thereof, and combinations of isocratic conditions with one or more of said gradients.

5. The method of claim 1, wherein the chromatography program is selected from
10 the group consisting of a high performance liquid chromatography program, a unified chromatography program, a high temperature high performance liquid chromatography program, a subcritical fluid chromatography program, a supercritical fluid chromatography program, and a hyperbaric chromatography program.

15 6. The method of claim 1, wherein step iii) further comprises calculating distance the solute peak travels during the given time segment and adding the distance to total distance the solute peak traveled.

7. The method of claim 6, further comprising the steps of:
20 vii) interpolating in the last time segment to estimate retention time of the solute peak.

8. The method of claim 7, further comprising:
viii) repeating steps i-vii) for at least one successive solute peak.

25 9. The method of any one of claims 1, 2, 3, 4, 5, 6, 7, and 8, wherein accumulated peak width at the given time segment is calculated according to an equation selected from the group consisting of:

$$\sigma_{\text{current total}} = \left(\left(\sigma_{\text{previous total}} * \left(1 - \frac{\frac{1}{1+k_{\text{current segment}}} - \frac{1}{1+k_{\text{previous segment}}}}{1 - \frac{1}{1+k_{\text{previous segment}}}} \right) \right)^2 + \sigma_{\text{current segment}}^2 \right)^{1/2}, \text{ wherein}$$

k represents retention factor, and σ represents peak standard deviation expressed as distance: algebraic equivalents thereof; an equation which can be transformed, using known identities from chromatographic theory, into an algebraic equivalent thereof; and
 5 derivations thereof wherein peak standard deviation is expressed as time or as volume.

10. A method for performing a multivariate optimization of a chromatographic separation, wherein the method comprises:

- i) developing a relation between peak retention and effective solvent strength for each solute in a chromatogram,
- ii) selecting a desired separation goal,
- iii) identifying more than one chromatographic parameter, and
- iv) searching through allowed values of the chromatographic parameters, and finding a combination of the values that produces the desired separation goal.

11. The method of claim 10, wherein step i) is carried out by developing a relation between $\log k$ and %B for each solute in a chromatogram, wherein k represents retention factor and %B represents volume percentage of a strong component.

12. The method of claim 11, wherein step i) is carried out by collecting data from two or more isocratic separations at different %B values, wherein the data comprise retention time for an unretained marker peak and retention time for at least one solute of interest, as a function of mobile phase composition; and thereafter regressing $\log k$ versus %B.

13. The method of claim 11, wherein step i) is carried out by collecting data from two or more gradient elution separations, wherein the separations are run at two or more different gradient rates, and wherein the gradient rates are linear, and thereafter estimating isocratic k values to derive the relation between $\log k$ and %B.

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14. The method of claim 10, wherein step i) is carried out by regressing any parameter affecting k values other than %B for some or all of the solutes and using the parameter in place of or in addition to %B.

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15. The method of claim 10, wherein the desired separation goal is selected from the group consisting of minimizing analysis time, minimizing solvent usage, minimizing cost of analysis, maximizing detectability of solutes, maximizing resolution within a given analysis time, maximizing resolution within a solvent usage limit, maximizing production rate of a solute at column outlet at a stated level of purity from other sample components, minimizing production cost, and combinations thereof.

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16. The method of claim 15, wherein step iv) is carried out by a method selected from the group consisting of full factorial analysis over the parameter values and coarse factorial analyses over more than one region of the parameter values.

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17. A method for performing a multivariate optimization of a chromatographic separation, wherein the method comprises:

i) storing a relation between peak retention and effective solvent strength for each solute in a chromatogram,

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ii) setting as a first default, a desired separation goal,

iii) setting as a second default, more than one chromatographic parameter, and

iv) searching through allowed values of the chromatographic parameters, and finding a combination of the values that produces the desired separation goal.

18. A method for modeling, predicting, and optimizing gradient elution high performance liquid chromatography separations, wherein the method comprises the steps of:

- 5 1) describing physical dimensions of a high performance liquid chromatography system;
- 2) collecting data from at least two isocratic separations, wherein the data comprise
 - a) retention time for an unretained marker as a function of mobile phase composition expressed as %B,
 - 10 b) retention time for at least one solute peak of interest as a function of mobile phase composition expressed as %B, and
 - c) mobile phase pressure,
 wherein the isocratic separations are carried out at different %B values;
- 15 3) developing a relation between retention time expressed as $\log k$ and %B for the solute peak of interest in step 2), wherein the relation is developed by regression of the data collected in step 2);
- 4) predicting effects of parameter changes on the retention time of the solute peak of interest by a time segmented numerical analysis process comprising
 - i) performing a time segmented numerical analysis,
 - 20 wherein, within a given time segment, a strong component is presumed present in an amount that is constant;
 - ii) calculating distance the solute peak travels along the column during the given time segment and adding the distance to total distance the solute peak traveled along the column;
 - 25 iii) incrementing the amount of the strong component to its next value in a successive time segment; and
 - iv) repeating steps i-iii) until the solute peak elutes;
- 5) predicting effects of parameter changes on peak widths of the solutes of interest using a modified time segmented numerical estimation approach comprising
 - 30 i) performing a time segmented numerical analysis,

wherein, within a given time segment, a strong component is presumed present in an amount that is constant;

ii) calculating contribution to broadening of the solute peak in the given time segment;

5 iii) correcting accumulated peak width for peak compression occurring when the amount of strong component relative to weak component changes during the chromatography program;

 iv) incrementing the amount of the strong component to its next value in a successive time segment; and

10 v) repeating steps i-iv) until the solute peak elutes;

6) determining the mobile phase pressure necessary at column inlet to sustain flow rates investigated in steps 4) and 5) from the pressure data collected in step 2); and

7) performing a multivariate optimization of user-adjustable chromatographic parameters, wherein multivariate optimization is carried out by a method comprising

15 i) selecting a desired separation goal,

 ii) identifying the chromatographic parameters,

 iii) searching through allowed values of the chromatographic parameters, and finding a combination of the values that produces the desired separation goal.

20 19. The method of claim 18, wherein more than one solute peak of interest is present and wherein the method further comprises repeating steps 2-6 for each successive solute peak before step 7).

20. The method of claim 18, wherein steps 4) and 5) are carried out concurrently.

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21. The method of claim 18, wherein the desired separation goal is selected from the group consisting of minimization of analysis time, minimization of solvent usage, maximizing detectability of the solutes, maximizing resolution within a given analysis time, maximizing resolution within a given solvent usage limit, maximizing production

rate of a solute at a desired level of purity from other components, and minimizing production cost.

22. The method of claim 18, wherein step 7iii) is carried out by method selected
 5 from the group consisting of a full factorial analysis in which the parameters are searched systematically at regular intervals over permissible ranges of all parameter values and coarse factorial analyses over more than one region of the parameter values.

23. A method for predicting high performance liquid chromatography separations,
 10 wherein the method comprises the steps of:

1) inputting data comprising

I) physical dimensions of a high performance liquid chromatography
 system;

II) data from at least two isocratic separations, wherein the data comprise

15 a) retention time for an unretained marker as a function of mobile phase composition expressed as %B,

b) retention time for at least one solute peak of interest as a function of mobile phase composition expressed as %B, and

c) mobile phase pressure,

20 wherein the isocratic separations are carried out at different %B values;

2) transmitting the data input in step 1) to an internet web site, wherein the web site generates results using the data to model, predict, and optimize the separation by a process comprising

25 I) developing a relation between retention time expressed as $\log k$ and %B for the solute peak of interest in step 1), wherein the relation is developed by regression of the data input in step 1);

II) predicting effects of parameter changes on the retention time of the solute peak of interest by a time segmented numerical analysis process comprising

i) performing a time segmented numerical analysis,

wherein, within a given time segment, a strong component is presumed present in an amount that is constant;

ii) calculating distance the solute peak travels along the column during the given time segment and adding the distance to total distance the solute peak traveled along the column;

iii) incrementing the amount of the strong component to its next value in a successive time segment; and

iv) repeating steps i-iii) until the solute peak elutes;

III) predicting effects of parameter changes on peak widths of the solutes of interest using a modified time segmented numerical estimation approach comprising

i) performing a time segmented numerical analysis,

wherein, within a given time segment, a strong component is presumed present in an amount that is constant;

ii) calculating contribution to broadening of the solute peak in the given time segment;

iii) correcting accumulated peak width for peak compression occurring when the amount of strong component relative to weak component changes during the chromatography program;

iv) incrementing the amount of the strong component to its next value in a successive time segment; and

v) repeating steps i-iv) until the solute peak elutes;

IV) determining the mobile phase pressure necessary at column inlet to sustain flow rates investigated in steps 4) and 5) from the pressure data collected in step 2); and

V) performing a multivariate optimization of user-adjustable chromatographic parameters, wherein multivariate optimization is carried out by a method comprising

i) selecting a desired separation goal,

ii) identifying the chromatographic parameters,

iii) searching through allowed values of the chromatographic parameters, and finding a combination of the values that produces the desired separation goal; and

3) receiving the results generated in step 2).

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24. The method of step 23, further comprising:

4) verifying the results by running a separation using the results received in step 3).

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25. An article of manufacture comprising:

signal bearing media embodying a program of machine readable instructions executable by a data processor to perform method steps for modeling a chromatography separation, wherein the method steps comprise: i) performing a time segmented numerical analysis, wherein, within a given time segment, a strong component is presumed present in an amount that is constant;

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ii) calculating contribution to broadening of a solute peak in the given time segment;

iii) correcting accumulated peak width for peak compression occurring when the amount of strong component relative to weak component changes during the chromatography program;

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iv) incrementing the amount of the strong component to its next value in a successive time segment;

v) repeating steps i-iv) until the solute peak elutes; and

vi) displaying the accumulated peak width of the solute peak.

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26. The article of claim 25, further comprising signal bearing media embodying a program of machine readable instructions executable by a data processor to perform a method step comprising: vii) repeating steps i-vi) for at least one successive solute peak.

27. The article of claim 26, further comprising signal bearing media embodying a program of machine readable instructions executable by a data processor to perform a method step comprising: calculating distance the solute peak travels along the column during the given time segment and adding the distance to total distance the solute peak traveled along the column.

28. The article of claim 27, further comprising signal bearing media embodying a program of machine readable instructions executable by a data processor to perform a method step comprising: interpolating in the last time segment to estimate retention time of the solute peak.

29. An article of manufacture comprising signal bearing media embodying a program of machine readable instructions executable by a data processor to perform method steps comprising:

i) developing a relation between peak retention and effective solvent strength for each solute in a chromatogram,

ii) storing a desired separation goal and identifying more than one operational parameters,

iii) searching through allowed values of the operational parameters, and finding a combination of the values that produces the desired separation goal.

30. An article of manufacture comprising signal bearing media embodying a program of machine readable instructions executable by a data processor to perform method steps for modeling a chromatography separation, wherein the method steps comprise

1) storing physical dimensions of a high performance liquid chromatography system;

2) collecting data from at least two isocratic separations, wherein the data comprise

a) retention time for an unretained marker as a function of mobile phase composition expressed as %B,

b) retention time for at least one solute peak of interest as a function of mobile phase composition expressed as %B, and

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c) mobile phase pressure,

wherein the isocratic separations are carried out at different %B values;

3) developing a relation between retention time expressed as $\log k$ and %B for the solute peak of interest in step 2), wherein the relation is developed by regression of the data collected in step 2);

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4) predicting effects of parameter changes on the retention time of the solute peak of interest by a time segmented numerical analysis process comprising

i) performing a time segmented numerical analysis,

wherein, within a given time segment, a strong component is presumed present in an amount that is constant;

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ii) calculating distance the solute peak travels along the column during the given time segment and adding the distance to total distance the solute peak traveled along the column;

iii) incrementing the amount of the strong component to its next value in a successive time segment; and

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iv) repeating steps i-iii) until the solute peak elutes;

5) predicting effects of parameter changes on peak widths of the solutes of interest using a modified time segmented numerical estimation approach comprising

i) performing a time segmented numerical analysis,

wherein, within a given time segment, a strong component is presumed present in an amount that is constant;

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ii) calculating contribution to broadening of the solute peak in the given time segment;

iii) correcting accumulated peak width for peak compression occurring when the amount of strong component relative to weak component changes during the chromatography program;

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iv) incrementing the amount of the strong component to its next value in a successive time segment; and

v) repeating steps i-iv) until the solute peak elutes;

6) determining the mobile phase pressure necessary at column inlet to sustain flow rates investigated in steps 4) and 5) from the pressure data collected in step 2); and

7) performing a multivariate optimization of user-adjustable chromatographic parameters, wherein multivariate optimization is carried out by a method comprising

i) selecting a desired separation goal,

ii) identifying the chromatographic parameters,

iii) searching through allowed values of the chromatographic parameters, and finding a combination of the values that produces the desired separation goal.

31. The article of claim 30, wherein more than one solute peak of interest is present and wherein the article further comprises signal bearing media embodying a program of machine readable instructions executable by a data processor to perform a method step comprising repeating steps 2-6 for each successive solute peak before step 7).

32. An article of manufacture comprising signal bearing media embodying a program of machine readable instructions executable by a data processor to perform method steps comprising:

1) developing a mathematical model of a process, wherein the mathematical model comprises a relation between at least two operational parameters,

2) identifying variables within the model that affect the relation,

3) selecting at least one desired end result,

4) searching through allowed values of the identified variables, and finding a combination of the values that produces the desired end result.

33. The article according to any one of claims 25-32, wherein the signal bearing media is selected from the group consisting of transmission type media, recordable media, and internet web sites.

34. A method for developing a high performance liquid chromatography protocol comprising the steps of:

- 1) collecting data from initial laboratory experiments,
- 5 2) developing a mathematical model to predict retention time and peak width of a solute peak, wherein the model relates retention to mobile phase strength,
- 3) predicting retention time and peak width using the model developed in step 2),
- 4) performing a multivariate optimization of user adjustable parameters affecting retention time and peak width, and
- 10 5) implementing the optimized parameters in a high performance liquid chromatography system.